SARATOGA SPRINGS PUPFISH
Cyprinodon nevadensis nevadensis (Miller)

Status: High Concern. Saratoga Springs pupfish numbers appear to be stable; however, they should be monitored closely because limited distribution in extreme habitats increases their vulnerability to anthropogenic and natural stressors.

Description: All Amargosa pupfish (*Cyprinodon nevadensis)* subspecies are small, rarely exceeding 50 mm TL. The body is deep, especially in reproductive males. The head is blunt and slopes steeply to a small, terminal, oblique mouth. There is one row of tricuspid teeth on each jaw, with the central cusps being truncated or pointed. *Cyprinodon nevadensis* is a variable species, but can be distinguished by the following morphometric characteristics: (1) the scales are large, the circuli lack spine-like projections, and the interspaces are reticulated; (2) there are 23-28 scales (usually 25-26) along the lateral line and 15-24 scales (usually 16-18) anterior to the dorsal fin; (3) the pelvic fins are reduced and may even be absent; (4) there are 8-11 anal fin rays (usually 10), 11-18 pectoral fin rays (usually 15-17), 0-9 pelvic fin rays (usually 6), and 14-22 caudal fin rays (usually 16-19); gill rakers range from 14-22 (usually 15-17) and preopercular pores from 7-17 (usually 12-14). Reproductive males in breeding colors are bright blue with a black band at the posterior edge of the caudal fin. Reproductive females are drab olive-brown and develop 6-10 vertical bars along the sides which may be distinct or faint. An ocellus (eyespot) is typically present on the posterior base of the dorsal fin of females.

Cyprinodon n. nevadensis can be distinguished from other subspecies by its deeper, broader body, anteriorly placed pelvic fins, and a greater average number of scales (Table 1). Scales are narrow and larger, with very dense and extensive reticulations and a high number of scale radii. Males of this subspecies have an intense blue coloration (Soltz and Naiman 1978).

Taxonomic Relationships: The fossil record and past geologic events suggest that the *Cyprinodon* species differentiated relatively recently, with most differentiation occurring during the pluvial-interpluvial fluctuations of the early to mid-Pleistocene (Miller 1981). Some differentiation may have occurred in the last 10,000 years, following the final recession of pluvial waters. As water table height receded in the Great Basin during the Pleistocene, numerous scattered lakes and streams shrank, isolating remnant populations of pupfishes, which led to allopatric speciation of *C. nevadensis*.

Cyprinodon nevadensis, the complex of subspecies commonly referred to as Amargosa pupfish, was first described from Saratoga Springs by Eigenmann and Eigenmann (1889). Following this initial description, the species was lumped with desert pupfish (*Cyprinodon macularius)* until Miller (1943) separated it out again. In subsequent studies, Miller (1948) recognized and described six subspecies of *C. nevadensis*, four of which occurred in California: the Saratoga Springs pupfish (*C. n. nevadensis),* the Amargosa River pupfish (*C. n. amargosae),* the Shoshone Spring pupfish (*C. n. shoshone),* and the Tecopa pupfish (*C. n. calidae*). Two more subspecies occur in Nevada: the Ash Meadows pupfish (*C. n. mionectes)* and the Warm Springs pupfish (*C. n. pectoralis*). *Cyprinodon n. calidae* is now extinct (Moyle 2002).

Measure/ Count	*C. n. amargosae*			*C. n. nevadensis*			*C. n. shoshone*		
	male		female	male		female	male		female
		ALL			ALL			ALL	
Standard length (mm)		36			40			34	
*Body width	256		265	274		269	231		229
*Head length		305			312			307	
*Head depth	330		304	367		343	331		311
*Head width	240		259	257		256	233		231
*Snout length		101			97			89	
*Mouth width		117			115			114	
*Mandible length		198			95			93	
*Anal origin to caudle base	338		346	394		362	371		355
*Caudle peduncle length	264		237	277		253	263		251
*Anal fin base length	116		105	111		105	108		101
*Anal fin length	330		304	227		195	217		190
*Pelvic fin length	98		89	95		87	90		77
Anal fin ray count		10			10			10	
Dorsal fin ray count		10			10			10	
Pelvic fin ray count		6			6			4	
Pectoral fin ray count		16			16			16	
Caudal fin ray count		18			17			18	
Lateral line scales		26			26			26	
Predorsal scale count		19			18			18	
Dorsal fin to pelvic fin scale count		11			10			9	
Caudal peduncle circumference scale count		16			16			15	
Body circumference scale count		27			25			23	

*Expressed as percent of standard length x 1000.

Table 1. Comparative average morphometrics and meristics of *Cyprinodon nevadensis* subspecies. Adapted from Miller (1948).

Life History: Pupfish inhabit a wide variety of habitats and exhibit many adaptations to thermal and osmotic extremes (Miller 1981). Optimal temperature for growth is 22˚C, with growth ceasing below 17˚C and above 32˚C. At optimal temperatures, growth is extremely rapid and fish reach sexual maturity within four to six weeks (Miller 1948). Such a short generation time enables small populations to remain viable. Generation time varies among the subspecies, with populations living in widely fluctuating environmental conditions exhibiting shorter generation times (Moyle 2002). Young adults (15-30 mm SL) of *C. nevadensis* usually constitute a majority of the biomass throughout the year (Naiman 1976). Reproductive activity in Saratoga Springs peaks during the spring, tapers off during the summer, and is virtually nonexistent during fall and winter. This produces an annual population cycle with a low of about 800 pupfish in March and a high of about 2700 in September (LaBounty 2003).

Saratoga Springs pupfish, like other spring-dwelling subspecies, exhibit different reproductive behaviors than riverine forms (Kodric-Brown 1981). Males of spring-dwelling subspecies establish territories over substrate with topographic complexity suited for oviposition. Both sexes are promiscuous and a single female may lay eggs in a number of different territories. The demersal eggs are sticky and thus adhere to substrates. Females may lay a few eggs each day (not necessarily on consecutive days) throughout the year. Territorial defense by males confers some protection of the eggs from predators, but otherwise parental investment is limited to gamete production (Kodric-Brown 1981).

Little additional work has been done on the biology of Saratoga Springs pupfish; for more general information on the biology of the Amargosa pupfish species complex, see the Shoshone Spring pupfish account in this report and Moyle (2002).

Habitat Requirements: Saratoga Springs is roughly circular, approximately 10 m in diameter, 1-2 m deep (Miller 1948) and has a soft sand and silt bottom through which the spring inflow enters (P.B. Moyle and J. Katz, personal observations 2010). The spring water is clear and temperature is a constant 28-29°C. The spring overflows into a pond and then into a marsh 4-6 ha in area, ringed by sand dunes. The marsh has a grassy bottom with substrate consisting of mud and sand. Water temperatures fluctuate in the marsh area according to daily ambient temperature and may vary from 4 to 49°C, depending on season. Pupfish are largely inactive from late November to late January in water temperatures less than 7-10ºC. During summer, peak activity is concentrated at temperatures of 31-35ºC (LaBounty 2003). Pupfish tend to avoid temperatures exceeding 35°C, selecting areas along shore in 40-50 cm of water and between 20 and 30°C, when possible. As temperatures rise above 35-38ºC, fish will burrow into the marsh mud and, as temperatures cool in the fall, roughly a third of the population has been observed burrowing into marsh substrates for thermal refuge. Pupfish will move from shoreline areas into marshy meadow habitats when disturbed.

Reproduction occurs at temperatures between 28-35ºC, and reproductive behavior and reproductive colors fade at 35-38ºC (Labounty 2003). Juvenile fish are found in the marsh but are absent from the main spring, suggesting that spawning occurs only in the marsh. In 1995, fish abundance in the marsh exceeded that in the spring-pool by as much as two orders of magnitude, and length-frequency distributions differed between the two habitats. The spring-pool population was always dominated by adults, whereas juvenile fish dominated the marsh population. Length-weight regressions also showed that body condition of spring-pool fish exceeded that of marsh fish (Sada 2003).

Distribution: *Cyprinodon n. nevadensis* occur naturally only in Saratoga Springs and its outflow marsh in Death Valley National Park, San Bernardino County, California. This spring is located at an elevation of 70 m and is tributary to the Amargosa River (Miller 1948). Saratoga Springs pupfish were also introduced into "Lake" Tuendae (an artificial, spring-fed pond) at Zyzzyx, San Bernardino County, where they became established (Turner and Liu 1976). Inadvertent introduction of mosquitofish (*Gambusia affinis*) and accidental draining of a portion of Lake Tuendae during restoration appear to have led to a significant decrease in the pupfish population (Hughson and Woo 2004). More recent surveys, however, indicate a persistent population (S. Henkanaththegedara, pers. comm. 2008).

Trends in Abundance: Comparison of survey data collected in 1966 and 1995 at Saratoga Springs indicates that this population is stable and occupies all available habitat. Pupfish abundance estimates were similar between the two studies, with 1966 abundance estimates ranging from 761 to 3833, and 1995 estimates from 686 to 2993 (Sada 2003). While no systematic population survey has been performed on the Lake Tuendae population, incidental capture of pupfish during surveys for tui chub in 2006 and 2008 suggest an increasing population. In 2008, 1500 pupfish were captured. These capture data indicate that Lake Tuendae remains a viable refuge population (S. Henkanaththegedara, pers. comm. 2008).

Nature and Degree of Threats: The major threat to Saratoga Springs pupfish is the possibility that its unique habitat may become dewatered due to predicted climate change impacts, coupled with increasing human demand upon groundwater aquifers in this region. Saratoga Springs and the other springs on the eastern side of Death Valley are partially dependent on regional groundwater movement through large, ancient aquifers that extend into central Nevada and western Utah (Dettinger et al. 1995, Riggs and Deacon 2002, Deacon et al. 2007).

Agriculture. Ground water pumping for irrigation, even at great distances, could affect flow in Saratoga Springs. Agricultural impacts in this region have decreased as water supplies are increasingly captured by urban areas.

Urbanization. In order to meet increasing water demand by the city of Las Vegas, the Southern Nevada Water Authority (SNWA) proposed mining large quantities of water from several different valleys within the groundwater basin (Southern Nevada Water Authority 2004). The potential impacts of distant aquifer pumping were exemplified at Devils Hole in Nevada, which had reduced water levels as a result of water being pumped for irrigation from the Ash Meadows flow-system aquifer. The U.S. Supreme Court (United States v. Cappaert 1977) protected Devils Hole and the Devils Hole pupfish (*Cyprinodon diabolis*) by ordering that pumping stop (Deacon and Williams 1991). After rising and then stabilizing for a number of years, the water level in Devils Hole is now dropping again, likely the result of groundwater pumping a considerable distance away (Bedinger and Harrill 2006). If water withdrawals in the Amargosa region continue to increase and Las Vegas proceeds with its planned withdrawals, it is very likely that flows in the Amargosa River and tributary springs will be greatly reduced or eliminated during dry years. Demand for water and flood control is also on the rise with increasing human development of Tecopa and the upper Amargosa Valley.

Recreation. While Saratoga Springs is located in an area seldom visited by Death Valley tourists, public access is allowed and its protection relies largely on voluntary compliance with park rules. Isolation in limited, fragile, habitat increases risk to Saratoga Springs pupfish through potential contamination and introduction of exotic species and pathogens.

Alien species. Although Saratoga Springs is in Death Valley National Park, it is accessible to the public and, therefore, is vulnerable to intentional introduction of alien fishes or invertebrates. Alien species may compete with or prey on pupfish or introduce disease. The decline of the Lake Tuendae population of *C. n. nevadensis* after introduction of western mosquitofish to the lake (Hughson and Woo 2004) may serve as an example of the consequence of alien introduction. Concurrent with the introduction of mosquitofish, Mojave tui chub (*Siphateles bicolor mohavensis)* in Lake Tuendae were found to be infected with Asian tapeworm (*Bothriocephalus achelognathii*) and a perennial algal bloom began. It is uncertain whether mosquitofish caused the algae bloom but they were almost certainly the tapeworm vector. It is not known if the tapeworm is deleteriously affecting the pupfish population.

	Rating	Explanation
Major dams	n/a	
Agriculture	Medium	Ground water pumping for irrigation even at great distances could affect flow in Saratoga Springs
Grazing	n/a	
Rural Residential	n/a	
Urbanization	High	Groundwater pumping by the city of Las Vegas has potential to intercept aquifer water flowing to Saratoga Springs
Instream mining	n/a	
Mining	Low	Mining was a prominent land use in the area but no known impact of abandoned mines on Saratoga Springs
Transportation	n/a	
Logging	n/a	
Fire	n/a	
Estuary alteration	n/a	
Recreation	Medium	Public access to isolated habitat increases risk
Harvest	n/a	
Hatcheries	n/a	
Alien species	High	Introduction of alien species into Saratoga Springs may threaten pupfish population

Table 2. Major anthropogenic factors limiting, or potentially limiting, viability of populations of Saratoga Springs pupfish. Factors were rated on a five-level ordinal scale where a factor rated "critical" could push a species to extinction in 3 generations or 10 years, whichever is less; a factor rated "high" could push the species to extinction in 10 generations or 50 years whichever is less; a factor rated "medium" is unlikely to drive a species to extinction by itself but contributes to increased extinction risk; a factor rated "low" may reduce populations but extinction is unlikely as a result. A factor rated "n/a" has no known negative impact. Certainty of these judgments is high. See methods section for descriptions of the factors and explanation of the rating protocol.

Effects of Climate Change: Although all pupfishes of the American Southwest are remarkably well adapted to the wide range of salinity and temperature characterized by their desert habitats, they are also remarkably vulnerable to change. Isolated desert springs and rivers fed by subsurface flow systems are precarious ecosystems, vulnerable to geologic and anthropogenic disruption. Fed by rain and snow melt at high elevation in the desert mountain ranges (Riggs and Deacon 2002), desert aquifers in the Death Valley region will likely receive less recharge as the region warms. Reduced recharge will be compounded by growing human demand for water in southern Nevada which already exceeds supply in this arid region. Predicted increases in air temperature may also have direct impacts on Saratoga Springs pupfish if water temperatures in the spawning habitat of Saratoga Springs Marsh exceed thermal tolerances for egg production. Moyle et al.

(2013) rated Saratoga Springs pupfish as "highly" vulnerable to extinction in the next 100 years due to climate change impacts.

Status Determination Score = 2.3 - High Concern (see Methods section Table 2). Isolated in a single spring system, the Saratoga Springs pupfish remains vulnerable to both anthropogenic and natural perturbations. NatureServe assigns the Saratoga Springs pupfish a conservation rank of "Critically Imperiled" (G2T1, http://www.natureserve.org/explorer/) due to small range and threats of hybridization and predation, while the American Fisheries Society considers it to be "Threatened" (Jelks et al. 2008). As a consequence of proposed aquifer pumping, the Superintendent of Death Valley National Monument (E. L. Rothfuss, letter to B. Bolster of CDFW, 27 May 1992) recommended the Saratoga Springs pupfish be listed as threatened. This recommendation was endorsed by the Desert Fishes Council (E. P. Pister, pers. comm.).

Metric	Score	Justification
Area occupied	2	Native range confined to Saratoga Springs with one small refuge population in Lake Tuendae
Estimated adult abundance	3	Population stable with short generation time
Intervention dependence	3	Continuous protection of spring required
Tolerance	2	Although remarkably adapted to temperatures and salinities that would kill most other fishes, Saratoga Springs pupfish exist at the very edge of their reproductive tolerances
Genetic risk	3	Single population but no apparent signs of genetic bottleneck or inbreeding depression
Anthropogenic threats	2	See Table 2
Climate change	1	Threatened by reduced recharge capacity in base aquifer and increases in temperature
Average	2.3	16/7
Certainty (1-4)	4	Small population easily monitored

Table 3. Metrics for determining the status of Saratoga Springs pupfish, where 1 is a major negative factor contributing to status, 5 is a factor with no or positive effects on status, and 2-4 are intermediate values. See methods section for further explanation.

Management Recommendations: The Saratoga Springs complex is protected by the National Park Service and is in near-pristine condition. However, flow reduction associated with groundwater pumping and increasing aridity remains a threat. Hydrological studies should be initiated to determine whether or not Saratoga Springs (and other springs in the region) is/are connected to the aquifer being pumped. A contingency plan should be developed that includes the identification of natural and/or artificial habitats to temporarily hold pupfish from Saratoga Springs in the event population loss appears imminent. The population at Zyzzxx should be monitored and maintained as a refuge population.

Figure 1. Distribution of Saratoga Springs pupfish, *Cyprinodon nevadensis nevadensis*, in California. Dashed grey circle represents refuge population at Zyzzxx (Lake Tuendae).